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Optogenetic intervention to the vascular endothelium

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ABSTRACT

Endothelium lining the interior of cardiovascular system and most visceral organs plays an important role in vascular function. Its dysfunction occurs in some of the most challenging diseases. An important function of the endothelium is to release vasoactive substances that act on the smooth muscle to change vascular tones. Substance secretion from endocrine cells relies on membrane potentials and firing activity, while it is unclear whether the membrane potential regulates substance release from the ECs. Understanding of this requires selective intervention to membrane potentials of the endothelial cells in situ. Here we show a novel intervention to endothelial cells using the optogenetic approach. A strain of transgenic mice was developed with the Cre–loxP recombination system. These transgenic mice expressed channelrhodopsin (ChR) in endothelial cells driven by the vascular endothelial cadherin or *cdh5* promoter. Linked in a tandem with YFP, the ChR expression was detected by YFP fluorescence in various endothelium-lining tissues and organs. The YFP fluorescence was observed in the lumen of blood vessels and pericardium, but not in tissues beneath the endothelium lining. Optostimulation of dissociated endothelial cells evoked inward currents and depolarization. In the isolated and perfused heart, surprisingly, optostimulation of endothelial cells produced fast, robust, reproducible and long-lasting vasoconstriction that was not blocked by either ET-1A or TXA2 receptor antagonist. Similar optical vasoconstriction was found in the isolated and perfused kidney. These results indicate that the optogenetics is an effective intervention to vascular endothelium where optostimulation produces vasoconstriction.

1. Introduction

Endothelium, a single layer of cell lining in the cardiovascular system, lymph vessels and several internal organs, plays a critical role in vascular function including substance exchange, vascular tone regulation, angiogenesis and thrombosis [18]. Endothelial dysfunction contributes to several cardiovascular diseases such as hypertension, shock, stroke and diabetic vascular complications [7].

The endothelium is a major tissue with approximately 10^{13} cells in an adult human [5], a number that is 100 times more than all neurons in the brain. The endothelium tissue not only is a mechanical barrier between blood and other tissues, but also resembles the endocrine system [16]. Indeed, a prominent function of endothelial cells (ECs) is to release vasoactive substances that act on the smooth muscle (SM) to produce vasoconstriction or vasodilation, maintaining a homeostatic state of vascular tones under different conditions. How the ECs selectively release vasodilators or vasoconstrictors is unclear.

Most endocrine cells are excitable, which secrete hormones and transmitters via depolarization and firing activity [21]. In contrast, ECs are nonexcitable. Whether the membrane potential regulates substance release from the ECs remains elusive. It is known that the membrane potential can affect a number of ion channels, transporters, intracellular Ca^{2+}, intracellular pH, etc. Activity of these molecules can in turn change cellular functions. Therefore, it is reasonable to believe that depolarization can affect the endothelium function. If such an assumption is proven correct, then it would be possible to reveal whether the endothelial depolarization results in vasodilation or vasoconstriction. To address these issues, novel methods are needed to selectively intervene to the membrane potentials of ECs when they remain interacting with vascular SM in situ, which will help to understand EC function in vascular tone regulation, and may have impacts on therapeutic designs for several cardiovascular diseases.

The successful development of optogenetics in neuroscience research [3,23] provides a unique way to access the endothelial membrane potentials with light. Generally, the optogenetics is a combination of genetics, electrophysiology and optics to control well-defined events within specific cells of living tissue by expressing an opsin in the cells. This approach allows to control membrane potentials and excitability of the opsin-expressing cells, which has been achieved in various types of neurons [27], glia [8], myocardium [2], skeletal muscle cells [24] and the vascular smooth muscle [28].

To demonstrate whether the EC function can be manipulated by optogenetics, we developed a new strain of mice that expressed channelrhodopsin (ChR) in ECs. The expression of ChR was detected in the lumen of blood vessels from multiple organs. Characteristics of optoactivation of ECs, including membrane excitability and vasomotor

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reactivity, were studied in the heart and kidney using combined optostimulation and traditional physiological techniques. Surprisingly, our results indicated that optostimulation of ECs can produce fast, reproducible, long-lasting vasoconstriction that is comparable in strength to the popular vasoconstrictor phenylephrine (PE).

2. Methods and materials

2.1. Generation of the cdh5–ChR transgenic mouse

All experimental procedures in the animal were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Georgia State University Institutional Animal Care and Use Committee. Transgenic cdh5–ChR mice with fluorescence labeled endothelium were generated by mating the *cdh5* promoter-driven Cre mice (B6.FVB-Tg(*Cdh*5–Cre)7Mlia/J, stock no. 006137; JaxMice) and cross-bred them with ChR–loxP mice (B6; 129S-Gt(ROSA)26Sor$^{tm32(CAG-COP4*H134R/EYFP)Hze}$/J, stock no. 012569; JaxMice). Animals were genotyped by PCR using punched ear or tail tissue. The presence of the Cre transgene (~100 bp) was detected using the following primers: Cre fw: 5′ GCGGTCTGGCAG TAAAAACTA TC and Cre re: 5′ GTGAAACAGCATTGCTGTCACTT, which would not distinguish hemizygous from homozygous mice. The presence of loxP was detected by using following primers with indication of ChR–loxP (212 bp), heterozygote (212 bp and 297 bp), or WT (297 bp): WT ChR fw: 5′ AAGGGAGCTGCAGTGGAGTA and WT ChR re: 5′ CCGAAAAT CTGTGGGAAGTC; ChR fw: 5′ ACATGGTCCTGCTGGAGTTC and ChR re: 5′ GGCATTAAAGCAGCGTATCC.

2.2. Histology of ChR labeled endothelium in multiple organs

Heart and kidney from cdh5–ChR and wild type (WT) mice were fixed in 1% paraformaldehyde at room temperature for over 4 h and then dehydrated in 30% sucrose in phosphate buffered saline (PBS) at 4 °C for 24 h. Fixed tissues were embedded in the Tissue-TekTM CRYO-OCT Compound (Andwin Scientific, Torrance, CA, 4583), and cut into 8–10 μm slices by using the Microm HM 550 Cryostats system (Thermo Scientific, PA, 22-050-337). Dura mater was peeled off from inner side of skull and mount on glassed slices directly. YFP fluorescence was detected with 514/527 nm (excitation/emission wavelength) filters under the microscope (Carl Zeiss, Gottingen, Germany, Axiovert 200).

2.3. Acute dissociation of aortic ECs

ECs were acutely dissociated from aorta obtained from both cdh5–ChR and WT mice by enzymatic dissociation for 10–15 min at 37° C using neutral protease (8 U/ml, obtained from Sigma, St. Louis, MO) and elastase (2 U/ml, Worthington, Lakewood, NJ, USA) mg/ml in the following digestion buffer (in mM): 138 NaCl, 5 KCl, 1.5 $MgCl_2$, 0.42 Na_2HPO_4, 0.44 NaH_2PO_4, 0.1 $CaCl_2$, 10 HEPES, 4.2 $NaHCO_3$ and 0.3% BSA. This was followed by a 1–2 min 37° C incubation with collagenase type IA (120 U/ml, obtained from Sigma, St. Louis, MO). The tissue segments were then washed with digestion buffer and gently triturated with a fire polished glass pipettes. The trituration solution containing ECs was dropped on a Petri dish coated with poly-L-lysine (Sigma, P8920) and easily identified via fluorescence microscopy.

2.4. Electrophysiology

Dissociated ECs were placed in the recording chamber and identified by YFP fluorescence using same microscope set up as described under histology section. Whole-cell currents and membrane potentials of the dissociated ECs were recorded at room temperature in the voltage and current clamp mode, respective. Recorded signals were amplified with an Axopatch 200B amplifier (Molecular Devices, Union City, CA), digitized at 10 kHz, filtered at 2 kHz, and collected with the Clampex 10 data acquisition software (Molecular Devices, Union City, CA). The patch pipettes with resistance of 4–6 MΩ were made with 1.2 mm borosilicate glass capillaries (Sutter Instrument CO., Novato, CA). The optical stimulation was delivered by using a xenon arc lamp with high-speed switcher (Lambda DG-4, Sutter Instruments, Novato, CA). The light source was connected to the incident-light illuminator port of the microscope, and passed through a 470 nm bandpass filter (~20 mW/mm^2). Light pulse trains were generated with the Digitimer D4030 pulse generator (Digitimer Ltd., Letchworth Garden City, UK). The solution applied to the bath contained (in mM) 130.0 NaCl, 10.0 KCl, 1.0 $MgCl_2$, 1.5 CaCl2, 10.0 glucose, 10.0 HEPES and 3.0 NaOH (pH 7.4). The internal (pipette) solution contained (in mM) 10.0 KCl, 133.0 K-gluconate, 5.0 EGTA, 5.0 glucose, 1.0 K_2-ATP, 0.5 Na-ADP, and 10.0 HEPES (pH 7.4), and the final Mg^{2+} concentration was adjusted to 1 mM using a $[Ca^{2+}]/[Mg^{2+}]$ calculation software Maxchelator (Chris Patton, Stanford University, Pacific Grove, CA).

2.5. Langendorff heart perfusion

Coronary circulation resistance was studied in the Langendorff isolated and perfused heart preparation. The heart and lungs were entirely removed from a terminally euthanized mice, and placed in the ice-cold Krebs–Henseleit (KH) solution containing (in mM): 119 NaCl, 4.7 KCl, 2.5 $MgSO_4$, 2.5 $CaCl_2$, 10 glucose, 0.5 disodium EDTA, and 25 $NaHCO_3$. All of pulmonary arteries and veins were tightened by cotton thread followed by removal of lungs. Then a cannula was inserted in the ascending aorta connected to the perfusion apparatus. This cannula is attached to the outflow of a reservoir containing an oxygenated KH solution, maintained at >~35 °C, and continuously gassed with 5% CO_2 and 95% O_2. The perfusion solution was delivered to coronary arteries in the retrograde direction through the aorta. After the perfusion speed was determined in each heart at a hydrostatic pressure (80 cm H_2O), perfusion at constant flow (0.2–0.4 ml/min) was carried out with a calibrated roller pump (Syringe Pump, Farmingdale, NY, NE-300, NE-4000). The perfusate exited the coronary venous circulation through the coronary sinus in the open vena cava. The viability of the isolated and perfused heart was assessed by its spontaneous beating (>250 beats/min), coronary vasoconstriction to 10^{-5} M PE and 60 mM KCl, and coronary vasodilation response to the β receptor agonist isoproterenol (Isop, 10^{-5} M).

2.6. Renal perfusion

Kidney from both cdh5–ChR and WT mice were isolated, cannulated through renal artery, and perfused at a constant rate (0.2–0.4 ml/min at baseline) with KH solution at 37 °C as described above. The viability of the isolated and perfused kidney was also assessed by renal vascular responses to 10^{-5} M PE, Isop, and KCl.

2.7. Data analysis

The electrophysiological data were analyzed with Clampfit 10.3 software. Data are presented as means ± SE. Student's t-test and one-way ANOVA were used to perform the statistical analysis. Difference was considered significant when $P \leq 0.05$.

3. Results

3.1. Generation of transgenic mice with ChR expression in ECs

To achieve endothelial expression of ChR, we took the advantage of two strains of commercially available mice, *cdh5* promoter-driven Cre mice (B6.FVB-Tg(*Cdh*5–Cre)7Mlia/J, stock no. 006137; JaxMice) [1] and ChR–loxP mice (B6; 129S-Gt(ROSA)26Sor$^{tm32(CAG-COP4*H134R/EYFP)Hze}$/J, stock no. 012569; JaxMice) [22], to generate a new strain of transgenic

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